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Surgical Wound Infection Caused by *Rahnella aquatilis*

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***Rahnella aquatilis* is a water-residing gram-negative rod, a member of the family *Enterobacteriaceae*, isolated rarely from clinical specimens of immunocompromised patients. A case of a surgical wound infection caused by *R. aquatilis* in a patient who underwent a prosthetic surgical intervention is reported. The presence of inducible β-lactamase was suggested by the disk induction test and the conventional agar dilution assay. Literature on *R. aquatilis* infections in humans is reviewed.**

Rahnella aquatilis is a gram-negative rod belonging to the family *Enterobacteriaceae*. In 1976, Gavini et al. (5) classified strains belonging or related to the genus *Enterobacter* by using numerical taxonomy and phenotypic characteristics and identified a new group in the family *Enterobacteriaceae*, which they named group H2. A DNA-DNA hybridization study, which was carried out in 1979 by Izard et al. (9), showed that there was close relatedness between the strains within group H2 but low genetic relatedness to other members of the *Enterobacteriaceae*. For this reason a new genus under the name *Rahnella* was introduced to include the species *R. aquatilis*. The generic name *Rahnella* was given to honor the German-American bacteriologist Otto Rahn, and the species name *aquatilis* was selected because the organism is usually isolated from water. *R. aquatilis* has rarely been associated with infections in humans (1, 2, 6–8).

In this paper we describe a case of surgical wound infection due to *R. aquatilis*, the biochemical characteristics, and the susceptibility of the microorganism to antibiotics.

CASE REPORT

A 63-year-old woman was admitted to the Emergency Care Unit of the University Hospital of Heraklion, Crete, Greece, in May 1991, with severe pain in her left knee, after she fell from a ladder. Radiologic examination showed a fracture of the left tibial condyles. Physical examination revealed pain in her left knee and moderate skin damage. The patient reported heavy consumption of alcohol and cigarettes (45 pack-years) and had a history of osteoporosis, which was treated with calcitonin.

Within the next day she underwent internal osteosynthesis (operative reduction with internal fixation). Eight days later, wound dehiscence occurred without obvious signs of inflammation. Cultures obtained at that time from the wound were negative. The wound was cleansed every day with hydrogen peroxide and an iodophor solution, and a gauze soaked with hypertonic saline solution was deposited inside it. The fixation materials were removed 45 days after the operation, and the wound was covered with free skin flap.

Seven days after the removal of the fixation materials and the plastic reconstruction, skin necrosis occurred and the wound remained uncovered. The size of the wound was 5 by 6 cm. Cultures obtained at that time were negative. The wound was cleansed every other day for a period of 8 months with hydrogen peroxide and an iodophor and was rinsed with hypertonic saline solution.

In February 1992, the wound was reduced in size but a purulent exudate appeared. A Gram stain of the purulent exudate revealed abundant polymorphonuclear leukocytes and a moderate number of gram-negative rods. Cultures of the pus yielded a pure growth of a gram-negative rod, identified as *R. aquatilis*. Two days later a second purulent specimen was obtained from the wound. Gram-stained smears revealed again abundant neutrophils and a few gram-negative bacteria. Culture of this second specimen grew again numerous colonies of *R. aquatilis*. The patient did not develop any fever. Four urine cultures and all four sets of blood cultures, obtained during a 40-day period following appearance of the purulent discharge, were sterile.

There was no clinical or radiologic evidence of osteomyelitis. On the basis of the bacteriologic findings, the patient was treated with intravenous trimethoprim-sulfamethoxazole (160 mg of trimethoprim and 800 mg of sulfamethoxazole every 8 h) for 15 days. The treatment was continued with the same dose every 12 h orally for another 30 days.

The infection responded well to the treatment, and the purulent discharge disappeared completely 10 days after the initiation of treatment. Cultures taken from the wound 5, 15, and 30 days after discontinuation of treatment did not reveal any microorganism. One month after discontinuation of treatment, a new skin grafting was performed with rotational free flap and the wound healed in a period of 20 days.

MATERIALS AND METHODS

The cotton swabs of the wound exudate, carried in Stuart transport medium, were cultured on the following media (all from BioMérieux S.A., Marcy l'Etoile, France) at 36°C for 48 h: blood agar (Columbia agar with 5% sheep blood), chocolate agar (Columbia agar with 2% hemoglobin and 1% IsoVitaleX), and Columbia CNA agar (Columbia agar with 5% sheep blood, colistin, and nalidixic acid) (all in 5% CO_2) and MacConkey agar and brucella agar with 5% sheep blood. The last medium was incubated anaerobically. Thioglycolate broth was also inoculated. The identification of the isolates was initially achieved by using the API 20E system (bioMérieux S.A.). Definitive identification was performed by conventional tube biochemical tests (4). Carbohydrate fermentation pattern of the microorganism was obtained by using phenol red broth base, in which carbohydrate solutions were added to a final concentration of 0.5% (for salicin) and 1% (for all others) (11).

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Antimicrobial susceptibility tests of the two isolates were initially performed by the disk diffusion method (12). The test results were confirmed with the National Committee for Clinical Laboratory Standards broth macrodilution procedure (13), determining the MICs of 20 antibiotics for the two *R. aquatilis* isolates.

A disk induction test (16) was performed, using a cefoxitin disk (30 μg) as the inducer and cefotaxime (30 μg) as the indicator. A conventional agar dilution assay (10) was also performed, determining the MICs of cefotaxime alone and in the presence of cefoxitin (100 μg/ml).

RESULTS

The bacterium grew well after 48 h of incubation at 36°C, in pure culture and in sufficient quantity, in the first and the second quadrant of streaked areas, on the following media: blood agar, chocolate agar, MacConkey agar, and brucella agar. The bacterium also grew in thioglycolate subculture to these media at 48 h. No growth was observed on Columbia CNA agar. Colonies were grey, smooth, and nonhemolytic on blood agar and small, red, and lactose fermenting on MacConkey agar.

Microscopy revealed a small gram-negative rod, which was subsequently identified as *R. aquatilis* by the API 20E system. The identification of both isolates was confirmed by the conventional biochemical tests listed in Table 1. The two strains were found to have an identical biochemical profile.

The isolates' phenotype to beta-lactam antibiotics was found to resemble that of *Enterobacter* species. The strains were also found to produce β-lactamase on nitrocefin disks (BBL Microbiology Systems, Cockeysville, Md.) and were resistant to ampicillin, amoxicillin-clavulanic acid, cephalothin, and cefoxitin. The isolates were susceptible to piperacillin, azlocillin, ticarcillin, cefamandole, cefotaxime, ceftazidime, ceftriaxone, aztreonam, imipenem, gentamicin, amikacin, tobramycin, netilmicin, trimethoprim-sulfamethoxazole, ciprofloxacin, and pefloxacin. The MICs of the antibiotics were equal for the two isolates (Table 2).

It was observed by the disk induction test that the isolates were induced to β-lactamase production by cefoxitin, in order to antagonize the activity of cefotaxime (Fig. 1). Because of the induction of β-lactamase among colonies residing in the area between the two disks, the zone of inhibition normally created by cefotaxime is truncated in the direction of the cefoxitin disk. In the agar dilution assay we found that low concentrations of cefoxitin (100 mg/liter) are sufficient to induce the production of β-lactamase. The MIC of cefotaxime was 0.5 mg/liter and increased to 32 mg/liter in the presence of cefoxitin.

TABLE 1. Biochemical reactions of the *R. aquatilis* isolates from this study compared with those reported in the literature

Test or characteristic	% Positive[a]	Reaction of our strains[b]
Motility		
36°C	6	−
22°C	100	+
Indole production	0	−
Methyl red	88	+
Voges-Proskauer	100	+
Citrate utilization (Simmons)	94	+
Hydrogen sulfide	0	−
Urea hydrolysis	0	−
Lysine decarboxylase	0	−
Ornithine decarboxylase	0	−
Arginine dihydrolase	0	−
Malonate utilization	100	+
o-Nitrophenyl-β-D-galactopyranoside	100	+
Phenylalanine deaminase	95	+[c]
Nitrate→nitrite	100	+
Oxidase, Kovács	0	−
Carbohydrate fermentation		
D-Glucose	100	+
Lactose	100	+
Maltose	94	+
L-Rhamnose	94	+
Raffinose	94	+
Salicin	100	+
Sucrose	100	+
D-Mannitol	100	+
Sorbitol	94	+
L-Arabinose	100	+
D-Xylose	94	+
Adonitol	0	−
Inositol	0	−
Mannose	100	+
Trehalose	100	+
Yellow pigment	0	−

[a] Percentage of *R. aquatilis* strains showing positive reactions as reported by Farmer et al. (4).
[b] −, negative; +, positive.
[c] Weak reaction.

DISCUSSION

It is possible that this case is a nosocomial infection caused by *R. aquatilis*. However, cultures of the solutions used for the cleansing and care of the wound and further epidemiologic investigation to detect the source of the organism were not performed.

Until now only seven clinical isolates of *R. aquatilis* have been reported. One strain was isolated from a burn wound (3). Harrell et al. (7) isolated *R. aquatilis* from the bronchial washing of a patient with AIDS. Christiaens et al. (2) recovered another strain of *R. aquatilis* from the sputum of a patient with chronic lymphocytic leukemia and emphysema. Two cases of *R. aquatilis* septicemia have been reported: the first in a patient with acute lymphocytic leukemia, diabetes mellitus, and bronchial asthma (6) and the second in a pediatric patient after bone marrow transplantation for neuroblastoma (8). Both infections were related to Hickman catheters. Alballaa et al. (1) described a urinary tract infection caused by *R. aquatilis* in a renal transplant patient. The clinical significance of the organism has been proven only in three of the above-mentioned cases; the two of septicemia and the other of the urinary tract infection. In all three cases the patients were immunocompromised. In the present case the isolation of *R. aquatilis* twice in pure culture from the pus of the surgical wound and the response of the infection to antibiotic treatment suggest clinical significance of the isolated organism.

The difference between the present case and the others mentioned above is that our patient did not have major immunosuppression. However, the surgical wound which did not heal for 13 months and the history of alcoholism may have resulted in impaired local resistance.

It is of interest that identification of *R. aquatilis* is quite difficult because of its resemblance to bacteria classified in the *Enterobacter-Erwinia herbicola* complex. The properties that differentiate *Rahnella* spp. are the weakly positive phenylalanine deaminase reaction, the absence of yellow pigmentation and the motility at 22°C but not at 36°C (14). Additionally, several commercial bacterial identification systems do not include *R. aquatilis* in their own databases, so strains of *R.*

TABLE 2. Antibiotic susceptibilities of the *R. aquatilis* isolates from this study

Antibiotic	MIC (mg/liter)
Ampicillin	>128
Piperacillin	4
Azlocillin	8
Ticarcillin	4
Amoxicillin-clavulanic acid	>128
Cephalothin	>128
Cefoxitin	>128
Cefamandole	4
Cefotaxime	0.5
Ceftazidime	0.5
Ceftriaxone	0.125
Aztreonam	0.125
Imipenem	0.125
Gentamicin	0.25
Amikacin	1
Tobramycin	0.25
Netilmicin	0.5
Trimethoprim-sulfamethoxazole	0.125/2.5
Ciprofloxacin	0.25
Pefloxacin	0.25

aquatilis may be misidentified as *Enterobacter agglomerans* (1). For this reason for the identification of our isolates, we did not use only the commercial systems but also the conventional tests as indicated by the relevant literature (4).

We have also noticed that the antibiogram of *R. aquatilis* to beta-lactams resembles that of the *Enterobacter* species in demonstrating resistance to ampicillin, amoxicillin-clavulanic acid, cephalothin, and cefoxitin with susceptibility to newer cephalosporins. Induction assays performed suggest that our isolates produce increased amounts of β-lactamase in the presence of an inducer. This is important in clinical practice because it may lead to the failure of therapy in patients receiving penicillins or cephalosporins which are β-lactamase inducers but are not highly stable to the enzyme produced.

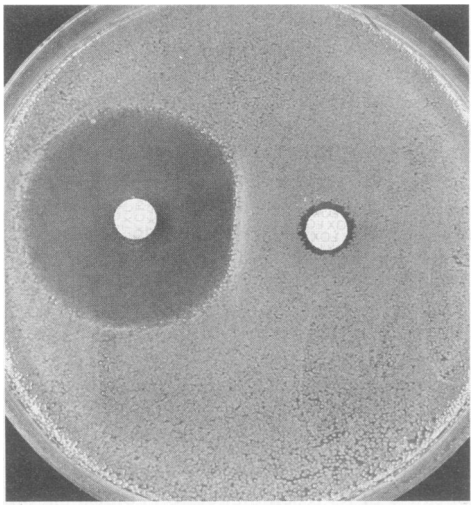

FIG. 1. Disk induction test indicating the antagonism between cefoxitin (FOX [30 μg]) and cefotaxime (CTX [30 μg]) against our strain of *R. aquatilis*.

β-Lactamase induction may also have clinical relevance when two beta-lactams are employed simultaneously, one of which is an inducer and the other of which is susceptible to hydrolysis by the induced enzyme (15). Further studies to identify the β-lactamase produced are in progress.

R. aquatilis is an organism which rarely causes infections. We believe that this case represents the first reported case of *R. aquatilis* surgical wound infection in an alcoholic patient.

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